PATENT

IRO-035A

IN THE UNITED STATES PATENT AND TRADEMARK FFICE

Applicant	:	Nabel et al.	Group Art Unit 1804
Appl. No.	:	08/210,902	
Filed	:	March 21, 1994	
For	:	INHIBITION OF ARTERIAL SMOOTH MUSCLE CELL PROLIFERATION	
Examiner	:	M. Newell	

DECLARATION OF ELIZABETH G. NABEL, M.D. UNDER RULE 132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Elizabeth G. Nabel, M.D., declare and state:

1. I have read the Office Action dated August 7, 1995, in the above-identified application.

2. I understand that the Examiner is of the opinion that based on the guidance presented in the specification, undue experimentation would be required to practice the claimed invention. As discussed at the Examiner Interview of October 26, 1995, inhibition of vascular cell proliferation using the method disclosed in the specification has been effectively demonstrated in three species: pig, rat and rabbit. In addition, expression of alkaline phosphatase using an adenoviral vector was shown to occur in both human blood vessels and atherosclerotic pig blood vessels.

These results are shown in Exhibit A which are true and correct copies of graphs and photographs shown at the interview and which accurately represent results achieved using the techniques disclosed in the specification. These results demonstrate that the invention works across species. The method of injury, adenoviral vector, mode of delivery, dosages and other parameters were the same in these experiments as described in Example 4, page 12 of the specification.

3. These results strongly support the conclusion that the same technique would effectively inhibit vascular cell proliferation in any mammal without undue experimentation. These results disprove or put to rest the doubts previously raised by the Examiner. None of our results support the Examiner's assertion of undue experimentation.

4. I also understand that the Examiner maintained the obviousness rejection over Takeshita et al. in view of Plautz et al. There is an established long felt but unsolved need for preventing restenosis in patients who have undergone balloon angioplasty. As stated by Muller et al., "conventional pharmacologic therapies directed at the inhibition of platelet activation, thrombus formation or vasoconstriction have failed to reduce the incidence of restenosis in clinical studies." (p. 460, Col. 2). The present invention satisfies this need.

5. It was unpredictable that useful inhibition of vascular cell proliferation could be effected by simply delivering the antiproliferative combination hsv-tk/ganciclovir to the vessel wall. Other antiproliferative agents have failed to accomplish this result. Enclosed herewith are three publications (Exhibit B) demonstrating the failure of three antiproliferative agents: methotrexate, cilazapril and angiopeptin, in effectively inhibiting vascular cell proliferation (Muller et al., *J. Am. Coll. Cardiol.*, 20:460-466, 1992; Lam et al., *Circulation*, 85:1542-1547; and Hong et al., *Circulation*, 88:638-648, 1993). Thus, in view of the failure of others, it was unpredictable that the instant method of inhibiting vascular cell proliferation using an adenoviral vector encoding the hsv-tk gene in combination with a DNA replication-inhibiting nucleoside analog, e.g., ganciclovir, would effectively inhibit vascular cell proliferation.

6. The success of the present method is unexpected. The induction of cell death using the claimed vascular cell proliferation-inhibiting method might be expected to result in a pro-inflammatory response, potentially leading to further injury and worsening of the restenosis. However, the instant method did not promote an inflammatory response.

7. It was also unexpected and unpredictable that the present method would successfully inhibit vascular cell proliferation despit a phenomenon called "washout". When DNA is instilled into a blood vessel, it is subjected to a strong fluid flow nvironment

once the catheter is r moved which would t nd to rapidly move away, or wash out, materials from the instillation site. In view of this "washout", it would not be expected that an instillation time of several minutes would result in entry and expression of an adequate amount of DNA into vascular cells to exert a proliferation-inhibiting effect. It was also unknown whether the instilled DNA would enter the cells responsible for excessive proliferation. However, even if the DNA entered the correct cells, it was not predictable that enough cells would be transfected to manifest a proliferation-inhibiting effect.

8. The present invention has received critical acclaim by others in the cardiovascular field. The enclosed article from *Science*, 265:738, 1994 (Exhibit C) describes the hsv-tk/ganciclovir experiments performed in the pig model and states that the result obtained in the pig model using hsv-tk/ganciclovir is "the most significant progress yet on the gene therapy front." This article also includes the praise of Stanford cardiologist Dr. Victor Dzau, who stated that the Nabels "have fulfilled the fantasy that gene therapy can work in this condition...and they have used a different [animal] model in the rat, one which allows extrapolation to humans."

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that thes statements were made with the knowledge that willful, false stat ments and the

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like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR

Dated: 11/06/95

By: Elizabeth G. Nabel
Elizabeth G. Nabel, M.D.

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